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# RESISTANCE TO MERCURY SALTS IN *Escherichia coli*

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## SYNOPSIS

The objective of study was to determine the in vitro susceptibility of *Escherichia coli* against mercuric chloride. Among *E. coli* tested strains the higher number (103) were susceptible to the 4 mg/l mercury concentration. Mercury was active in terms of MIC 50 (2 mg/L) against 41.47% of strains. According to the MIC susceptibility breakpoints (4 mg/L) the resistant strains (175) was approximately two fold lower to that of susceptible strains (n=300).

## INTRODUCTION

The heavy metals have been used in medicine at the ancient times. Mercuric chloride was used as an antiseptic wound application and was introduced by Arab physicians.

In the 19$^{th}$ century mercury compound have been developed parallel with the scientific identification of microbial species and the implication of microorganisms with a specific disease. Other chemical compounds were introduced after 1900 years, but later, in 1950 period their use was progressive restraint only as disinfectants or preservatives.

In the 1970 period these compounds were also extensive use in the agriculture, but, the pollution environmental problems has been determinate the limitation for use. Mercury and its compounds are used in medicine as bacteriostatic and fungistatic agents. Many of the chemical forms of mercury are toxic to living organisms because of their affinity for cysteine residues in proteins (Osborn et al., 1997). Bacteria have genetically mechanisms for resistance to inorganic mercury with an important role in the cycling of these compounds in the natural environment.

Mercury resistance, encoded by the genes can be located on plasmids and transposons, was due to the action of two mercury-detoxicating enzymes, organomercurial lyase and mercuric reductase that covert toxic mercury to the less toxic metallic mercury and its vaporization (Foster, 1983; Russell, 1992). The use of organomercurials in liquid detergents and disinfectants promote resistance to mercury among bacteria from both natural and clinical environments (Porter et al., 1982). Dental amalgam and industries using mercury and its compounds are a source of human exposure to mercury vapor distributed in the atmosphere (Edlund et al., 1996).

The objective of this study was to determine the *in vitro* susceptibility of *Escherichia coli* strains against mercuric chloride.

## MATERIALS AND METHODS

BACTERIAL STRAINS. The organisms used in this study were 475 *E. coli* isolated during 2003-2008 period from urine.

The bacteria were isolated and identified according to the standard procedures used in microbiological laboratories.

SENSITIVITY TESTING. Minimum inhibitory concentrations (MICs) were determined by Mueller-Hinton agar dilution technique, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2008).

An inoculum in sterile saline of fresh overnight cultures, with a final concentrations about of $10^4$ colony formatory units per spot was delivered by a multipoint inoculator to a series of agar plates which obtained the antimicrobial agent in two fold dilutions (from 0.25 to 128 mg/L) (Ericson & Sherris, 1971).

The MICs were recorded after 24 h of plates incubation at $35^0$C as the lowest concentration of antimicrobial agent that inhibited the development of visible bacterial growth or growth ≤ 5 colonies was observed.

*Escherichia coli* American Type Culture Collection (ATCC) 25922 was included as MIC reference strain.

For the analysis of the results breakpoints proposed for resistance (≥16mg/L) and sensitivity (4mg/L) were used. Resistance rates are reported using the breakpoints for the susceptible category.

## RESULTS AND DISCUSSION

In total 475 strains were examined. In Table 1. the distribution of MIC values and the cumulative percentages of MIC of mercury for the *E. coli* strains investigated are shown. The range of MICs, MIC 50, MIC 90 values resistance percent and mean S-values for mercury are given in Table 2.

**Table 1: *In vitro* activity of mercury chloride against *E. coli* tested strains.**

| | MIC concentrations (mg/L) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 |
| No. of isolates with MIC (mg/L) | 5 | 40 | 77 | 75 | 103 | 70 | 63 | 21 | 21 |
| Percentage of inhibition | 1.05 | 8.42 | 16.21 | 15.78 | 21.68 | 14.73 | 13.26 | 4.42 | 4.42 |
| Cumulative percentage of inhibition | 1.05 | 9.47 | 25.68 | 41.47 | 63.15 | 77.89 | 91.15 | 95.57 | 100 |

The MIC ranged from 0.25 to 64 mg/L for *E. coli* isolates. Among *E. coli* tested strains the higher number (103) were susceptible to the 4 mg/L mercury concentration (Table 1).

Mercury was active in terms of MIC 50 (2 mg/L) against 41.47% of strains. This species is poorly susceptible to lower mercury concentrations. Only 9.47% of strains are inhibited at 0.25 mg/L and 0.5 mg/L respectively of mercury concentrations. The MIC 50 is a value which usually indicates the maximum of strains in one species showing an MIC around this value. The MIC 90 values of mercuric chloride against *E. coli* isolates was 16 mg/L (91.5%). This value is 4 – fold superior than MIC 50 (Table 2).

According to the MIC breakpoints (4 mg/L) the number of resistant strains (175) was approximately two fold lower to that of susceptible strains (n=300).

The mean S-values express the mean MIC of the susceptible isolates by excluding the strains with acquired resistance. This mean, that in our case has a value of 2 mg/L, is the best indicator of the intrinsic activity of the agent.

**Table 2: *In vitro* activity of mercury chloride against *E. coli* isolates.**

| Strains (475) | MIC range | MIC50 (mg/L) | MIC90 (mg/L) | % Resistance | Mean S-values |
|---|---|---|---|---|---|
| *E. coli* | 0.25-32 | 2 | 16 | 36.8 | 2.20 |

The mechanism of mercury compounds action results from the bind to the thiol groups of bacterial enzymes and other thiol radicals in microbial cells. Mercury compounds affect also and the mammalian cells because and these containe thiol radicals.

The increasing levels of bacterial resistance are a major problem complicating the use of antibacterial agents. The mechanisms of resistance are essentially of two types: intrinsic, a natural chromosomally controlled property of an organism and acquired through genetic changes in a bacterial cell and arising either by mutation or by the acquisition of genetic material from another cell via plasmid or transposon. Bacterial resistance to heavy metals is sometimes, but not invariably, an association with antibiotic resistance. Mercurial compounds, such as phenyl mercuric nitrate and acetate are used as antiseptic disinfectant and preservative agents in pharmaceutical products and thiomersal in biological products (bacterial and viral vaccines). In *E. coli* the plasmids conferring resistance to mercury and organomercury compounds are broad-spectrum (Foster, 1983).

## CONCLUSIONS

Reading the mercuric chloride activity, the results show a level resistance percent of 36.8% in *E.coli* strains.

Mercury released from silver amalgams and the use of organomercurials in liquid detergents and disinfectants may promote resistance to mercury among bacteria in hospitals, community and environment.

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